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The Short-term Exposure Effect of Chlorpyrifos (20% EC) on Haematological, Biochemical and Histopathological Response of Striped Catfish *Pangasianodon hypophthalmus*

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Abstract

An experiment of acute toxicity was conducted to find out the lethal concentration 50 (LC_{50}) of chloropyrifos (CPF) for *Pangasianodon hypophthalmus*. Our findings indicated that the 96-h LC_{50} value of CPF for *P. hypophthalmus* was 0.106 mg L^{-1}. Effects of different concentrations of CPF (0.09, 0.1, 0.11, 0.12, and 0.13 mg L^{-1}) for 96-h on hemato-biochemical, enzyme and histological effects were evaluated in this study. Acute exposure induced significant differences in enzymes acid phosphatase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and acetylcholine of blood serum of the treated group as compared to control group. However, significant decreases in serum total protein, globulin, albumin, and triglycerides levels while serum glucose enhanced with consequent increasing doses of CPF exposure. Also, CPF induced significant declines in RBC count, haemoglobin and hematocrit level whereas the exposure groups showed significant rises in WBC and blood indices levels as compared to control group. Furthermore, CPF exposure causes gill curling, epithelium lifting, necrosis, and lamellae degeneration. Hemosiderin, sinusoid dilations, necrosis, malanomacrophage, hepatocyte hypertrophy were observed in liver histology. Reduction of lumen tubule, hyperplasia, increased bowman space and degenerations were seen in kidney. Overall findings indicates that the acute CPF exposure to *P. hypophthalmus* resulted in significant adverse changes in biochemical, hematological, and histopathological response.

Introduction

The development and widespread use of pesticides, designed to combat dangerous parasites and diseases while benefiting crops, have inadvertently led to the contamination of aquatic systems (Chibee et al., 2021; Tharmavaram et al., 2021). Residues of pesticides enters aquatic ecosystem through multiple pathways, some of them are agricultural and urban runoff, spray drift and direct application. This has led to concentrations exceeding the toxicity levels for non-targeted vertebrate and invertebrate in densely populated areas (Yancheva et al., 2022).

In India, an organophosphate insecticide named Chlorpyrifos (CPF), widely used for agricultural purposes is posing a significant threat to aquatic ecosystems. CPF functions by inhibiting or suppressing the (AChE) activity to break the acetylcholine (Ach) in synaptic cleft and there by disrupting synaptic transmissions. IUPAC name for Chlorpyrifos is O,O-diethyl-O-(3,5,6-trichlor-2-pyridyl) phosphorothioate. CPF is toxic to fish, affecting their physiological functions, growth and reproduction functions (Levin et al., 2004; Sledge et al., 2011), also causing damage to the gills and liver (Xing et al., 2012). The LC_{50} of 96-hour (lethal concentration for 50% of the tested organisms) value for CPF (Chlorpyrifos), as

reported by Huang et al. in 2020, ranges between 0.5–15.395 µg L^{-1}. This indicates that even at relatively low concentrations, CPF can be highly toxic to fish species.

In the toxicity studies, haematological indices are studied for evaluating the fish's physiological status. Alterations in hematological parameters such as red blood cells, white blood cells, and plasma and serum levels result in histological abnormalities affecting the liver, kidneys, gills, muscles, brain, and gut

Variations in hematological parameters such as RBC, WBC, plasma and serum level results in histological changes which affects the gills, liver, kidney, brain, gut and muscles of various fish species when exposed to pesticides of different kinds (Tahir et al., 2021). The physiological changes which take place in organisms due to pathological or chemical stress can be predicted with the help of bloods biochemical profile (Nemcsok and Boross 1982).

The striped catfish, *Pangasianodon hypophthalmus*, stands out as one of the most widely distributed freshwater fish species. Renowned for its rapid growth rate, environmental tolerance, ability to survive under high stocking densities, simplicity of cultivation and rearing, ease of seed production, popularity among consumers, and advantages in long-distance transport in live conditions, *P. hypophthalmus* has experienced rapid cultural spread (Islam et al., 2019).

This present study seeks to assess the toxicological risk posed to *P. hypophthalmus* by investigating hematological and biochemical parameters, enzyme activity expression, and histopathological responses. The aim is to comprehensively figure out the impact of pesticide exposure on physiological and biochemical aspects of *P. hypophthalmus*. Findings of this research will help us to better understand the potential risks of pesticides on aquatic ecosystems, particularly concerning an economically and culturally important species.

Materials and Methods

Collection and Maintenance of Animals

Uniform sized fingerlings of striped catfish (Length 8±1 cm, Weight 4.8±0.9 g) were procured from the local supplier in Ratnagiri, Maharashtra. These fingerlings were acclimatized in a 500L FRP tank for a period of 15 days before the start of the experiment. The experiment was conducted at the wet laboratory of Aquaculture Department, College of Fisheries, Ratnagiri. Before exposure, fish spent at least 15 days acclimating to laboratory conditions with a natural photoperiod (12h: 12h L/D). According to APHA (2005), the physicochemical properties of the tap water utilised in this investigation were temperature 26±1.0°C, pH 7.4±0.06, DO 6.4±0.8 mg L^{-1}, and alkalinity 56.0±0.8 mg L^{-1}. Fish were fed commercial feed with 30% crude protein which is manufactured by Taiyo Feed Mill Pvt. Ltd. Tamilnadu, India (4% b/w twice a day) throughout the acclimatization period. The fish were starved for one day before start the experiment, and no feed was supplied during the trial.

Acute Toxicity Test

A total of 180 uniform size fish were used to analyze the acute toxicity. The bioassay experiment was carried out in rectangular glass aquaria (50 L capacity). Commercial Chlorpyrifos (O, O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate) 20% EC was (Registration no. CIR-16,143/93-CHLORPYRIFOS (EC)-388) purchased from G P Agro Agencies, Ratnagiri, Maharashtra, India and manufactured by Gharda chemicals limited, Mumbai, India. Trials were carried out at 0.09, 0.1, 0.11, 0.12, and 0.13 mg L^{-1} and LC_{50}-96 h value was determined. The test was carried out in triplicates for each concentration, with 10 fish in each replicate. The mortality (%) was measured every 24 hours and the dead fish were immediately removed. Fishes were not fed and application of a static non-renewable method was done (Reish and Oshida, 1986). Using the statistical programme SPSS 16.0, probit analysis was used to process the experiment's data.

Collection of Blood for the Assay

Samples of blood were collected from the fish that survived the 96-hour exposure to CPF. Fishes were randomly selected from control and experimental tanks for blood collection. Clove oil was used to anesthetize the fishes (Novelty Pharma Products, India, @ dose of 50 µl L^{-1}) before drawing blood (Karmakar et al., 2021). Blood was collected by puncturing the posterior caudal vein using a 1.0 ml disposable insulin syringe. Blood was transferred to a non-heparinized microcentrifuge tube to measure biochemical parameters and enzyme activity. After centrifuging the serum at 3000 g for 15 minutes to separate them, they were stored at -20°C until needed (Adel et al., 2017).

Hematological Analysis

Total erythrocyte count (TEC) was performed using improved Neubauer's double counting hemocytometer with RBC diluting fluid (Shah and Altindag, 2004). Erythrocyte was counted in the loaded haemocytometer chamber and total numbers were recorded as $\times 10^6$ mm^{-3} (Wintrobe, 1967). White blood cells (WBC) or total leucocyte count (TLC) were counted using leucocyte dilution fluid (Qualigens) in hemocytometer's Neubauers counting chamber according to Shaw method (1930). The Cyanomethemoglobin technique calculates haemoglobin percentage (Dacie and Lewis, 1995), while Snieszko's micro-hematocrit method (1960) determines hematocrit (PCV). Dacie and Lewis (1991) standard formulas are used to determine derived haematological indices of mean corpuscular volume,

mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.

$$MCV = (Hct/RBC) \times 10$$

$$MCH = (Haemoglobin / RBC) \times 10$$

$$MCHC = (Haemoglobin / Hct) \times 100$$

Serum Enzyme Assay

The Acid phosphatase (ACP) and Alkaline phosphatase (ALP) activity were estimated by using the method given by Walter and Schutt (1974). Similarly, Reitman and Frankel (1957) method was used to estimate the serum aspartate aminotransferase (AST) activity. To 1 ml of buffered aspartate about 0.2 ml of serum sample was added and this mixture was incubated for 60 min at 37°C. Later this mixture was allowed to stand at room temperature for 20 min after addition of 1 mL of 2,4- dinitrophenylhydrazine (2,4-DNPH). Thereafter this mixture was supplemented with 10 mL of 0.4 N NaOH, mixed well and set for 10 min before measuring at 505 nm. Serum alanine aminotransferase (ALT) activity was also assessed using the procedure of Reitman and Frankel (1957). Buffered L-alanine solution of 1 ml was added to 0.2 mL of serum sample and then it is incubated at 37°C for 30 min. Later this mixture was added with 1 mL of 2,4-DNPH and set at room temperature for 20 min. This mixture was added with 10 mL of 0.4 N NaOH, mixed well and measured at OD of 505 nm after allowing it to stand for 10 min. The AChE activity assay was conducted using Ellman et al., 1961 colorimetric method, using a cuvette with cholinergic iodide (0.015 M) and dithiobis nitrobenzoic acid (0.01) as substrates together with 0.1 M phosphate (8.0 pH). AChE activity was detected at 405 nm for 180 s.

Serum Biochemical Assay

Enzymatic colorimetric test by Mendel et al. (1954) was used to estimate the glucose levels. Total proteins in the serum were measured by Lawery's method as Glutamate pyruvate Bergmeyer (1974). The methods of Johnson et al., (1999) and Bucolo and David (1973) were used to determine the albumin (Alb) and total triglyceride (TG) levels, respectively. Globulin content was calculated by subtracting albumin values from the total serum protein.

Histopathological Study

Following a 96-hour acute toxicity exposure to CPF, collection of gill, kidney and liver tissue samples were done. Post collection a small section of liver, kidney and gill were fixed in 10% neutral buffer formalin (NBF) in 2 ml for 24-48 hours. Before the dehydration processing the samples were kept in 70% ethyl alcohol for overnight. To dehydrate the tissue samples, they were placed at various degrees of alcohol. Dehydrated samples of tissue were then embedded in paraffin to form the block, which was then kept at 4°C for 24 hours. Tissue slices (6m) were prepared using microtome (Leica RM2255, USA). After being rehydrated in graded alcohol, the slices of tissue were stained using haematoxylin and eosin (H & E), and examined under the microscope (Carl-Zeiss-Promenade 1007745 Jena, Germany). The histopathological assessments were conducted following Bullock (1989) and Karmakar et al. (2021) instructions.

Statistical Analysis

Data were shown as mean ± standard error (SE). Probit analysis (Regression Y= a + bX + e) was used to determine the LC_{50} value of CPF. One way ANOVA was used to analyze the data by using SPSS version 16.0 for Windows (SPSS Inc., USA). To find out the significant differences among the means ($p<0.05$), Tukey's post hoc test was used.

Results

Median Lethal Concentration

The LC_{50} value of CPF was estimated to be 0.1060 mg L^{-1} (0.09 to 0.13 mg L^{-1}) in *P. hypophthalmus* for 96 h by probit analysis (Finney, 1971). At the control (0.0 mg L^{-1}), there was no mortality for 96 hours (Figure 1).

Haematological Studies

The acute toxicity of chlorpyrifos exposure on hematological parameters of striped catfish was presented in Table 1. RBC count, the Hct level and the Hb concentration all significantly decreased during haematological assessment. In contrast, WBC, MCV, and MCH values increased as compared to the corresponding control groups.

Serum Enzyme Activity

Serum ACP, AST and ALT activities (Figure 2, Figure 3 and Figure 4) of *P. hypophthalmus* significantly spiked on exposure to CPF in comparison to control group at 96 h while ALP activities decreased gradually in fish as CPF concentrations increased (Figure 5). Inhibition of AChE activity (Figure 6) was observed in all the CPF 0.09 to 0.13 mg L^{-1} exposed treatments. At different sampling intervals a significant decrease in the activity of serum was recorded in fishes exposed to CPF.

Biochemical Analysis

Fish exposed to chlorpyrifos showed a significant increase in serum glucose levels and a decline in total protein content, globulins, albumin and triglyceride

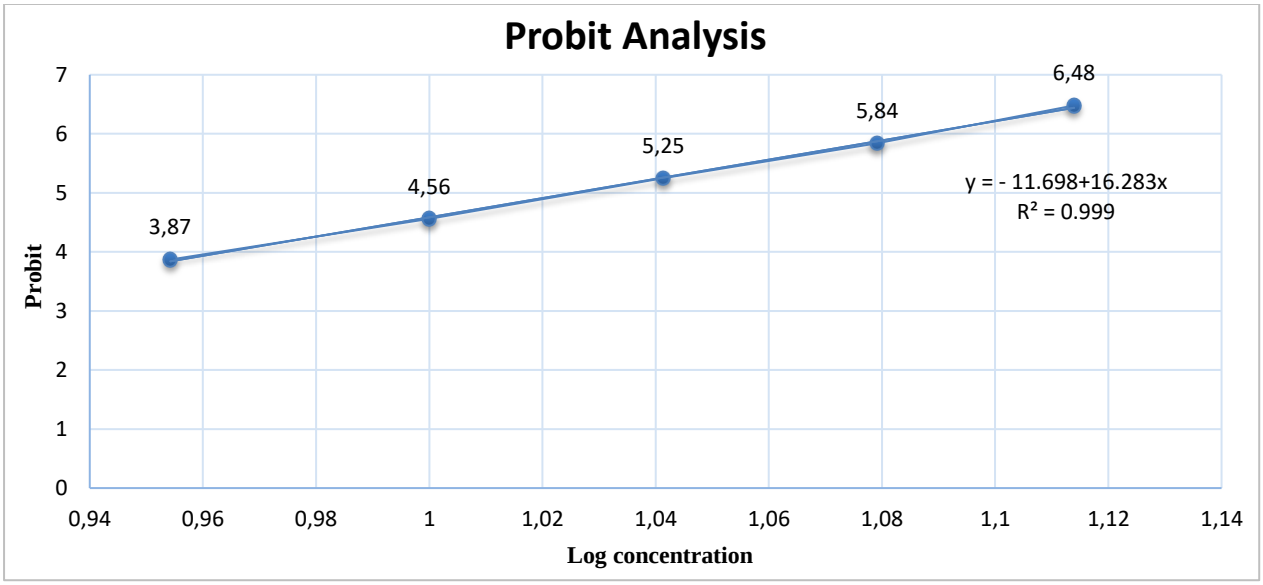

Figure 1. Linear relationship between mean probit mortality and log concentration of *Pangasianodon hypophthalmus* fingerlings exposed to acute concentrations of chlorpyrifos for 96 hours.

Table 1: Haematological parameters of *Pangasianodon hypophthalmus* exposed to different concentrations of chlorpyrifos for 96 hours.

Treatment/ parameter	RBC ($\times10^6$/mm^3)	WBC ($\times10^3$/mm^3)	Hb (g/dL)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control	2.26±.01^a	12033±145.30^a	12.93±.09^a	30.00±.06^a	132.55±.71^{cd}	57.14±.17^a	43.11±.35^{bc}
T1	2.03±.04^b	14333±88.19^b	11.77±.09^b	28.36±.03^b	140.06±2.42^b	58.11±1.44^a	41.48±.32^c
T2	1.72±.01^c	16567±120.19^c	10.30±.11^c	26.73±.12^c	155.43±.42^a	59.89±1.07^a	38.53±.60^d
T3	1.66±.01^c	18067±384.42^d	9.70±.10^d	22.90±.12^d	138.23±.89^{bc}	58.56±.53^a	42.35±.27^c
T4	1.51±.01^d	28033±145.30^e	9.03±.09^e	19.43±.07^e	128.42±.79^d	59.69±.38^a	46.48±.35^a
T5	1.40±.02^e	32733±233.33^f	8.17±.08^f	18.17±.07^f	129.78±1.16^d	58.34±.65^a	44.96±.64^{ab}

Values in the same column with different superscript differ significantly ($p<0.05$). Data expressed as Mean ± SE (n=6).

levels in comparison to the control groups. Dosage dependent effects were observed, lower protein content and higher glucose levels were recorded at higher chlorpyrifos concentration (Table 2).

Histological Alterations

Histological sections of the gills in the control group revealed no morphological anomalies throughout the experimental period (Figure 7A). In 0.09 mg L^{-1} CPF treated group, there were evidences of secondary lamellae curling, epithelium lifting, and hyperplasia in epithelium of secondary lamellae (7B). Meanwhile, the 0.1 mg L^{-1} CPF treatment exhibited curling of secondary lamellae, epithelial hyperplasia in primary lamellae, and rounding of secondary lamellae (7C). Furthermore, the 0.11 mg L^{-1} CPF treated group displayed more extensive damage in gill structure, including desquamation of secondary lamellae and thickening of the primary lamellar epithelium (7D). The histopathological alterations were notably more in 0.12 mg L^{-1} CPF treated group, revealing a shortening of telangiectasia (T) and secondary lamellae, secondary lamellar degeneration and increased blood congestion (7E). However, the 0.13 mg L^{-1} CPF treated group exhibited even more pronounced effects, including necrosis, collapsed secondary lamellae, and massive lamellae degeneration (7F).

The impact of acute chlorpyrifos (CPF) exposure on histological changes in the liver of striped catfish is depicted in Figure 8 (A-F). The morphological alterations in liver structure manifest gradually with increasing concentrations. The control group exhibits a normal liver structure (Figure 8A). In the 0.09 mg L^{-1} CPF-treated group, there are minor fatty changes, hemosiderin deposition, and mild cytoplasmic vacuolation (Figure 8B). The 0.1 mg L^{-1} CPF-treated group shows slightly enlarged nuclei, dilation of sinusoids, and fatty vacuolation (Figure 8C). At 0.11 mg L^{-1} CPF treatment, there is evidence of nuclei shrinkage, necrosis, hepatocyte vacuolation, and aggregation of melanomacrophages (Figure 8D). The 0.12 mg L^{-1} CPF-treated group displays irregular hepatic plates, karyolysis, cloudy swelling, and mild blood congestion (Figure 8E). At 0.13 mg L^{-1} CPF-treated group, there were irregular hepatic plates, massive necrosis, pyknotic nuclei, hypertrophy of hepatocytes, and large vacuolations (Figure 8F).

After acute chlorpyrifos (CPF) exposure, histopathological examination of the kidneys of *P.*

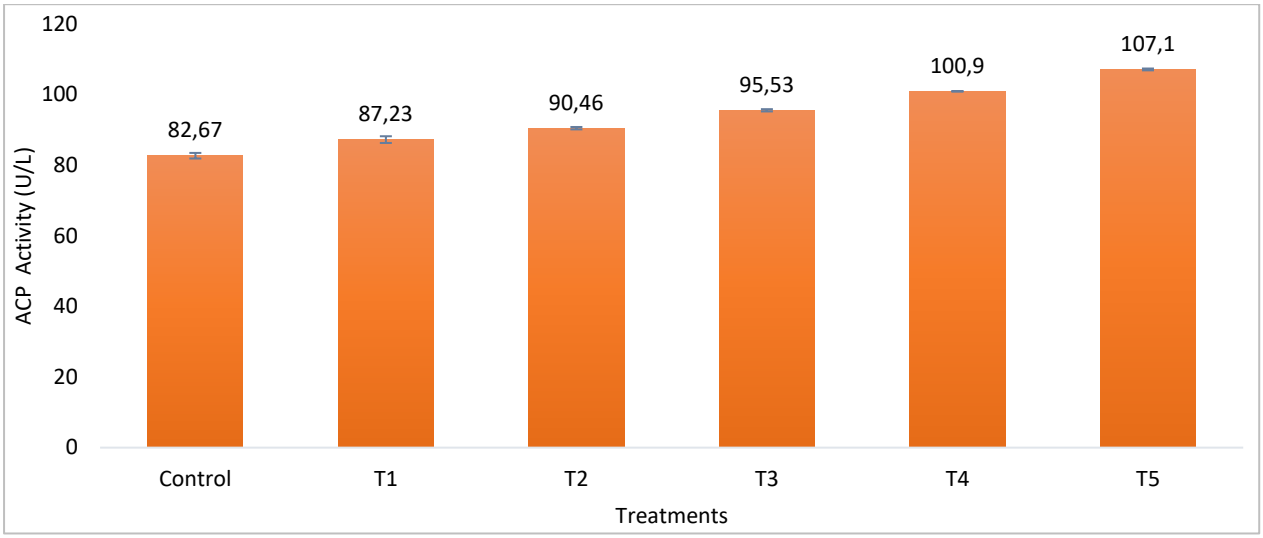

Figure 2. The effects of chlorpyrifos on the Serum ACP levels of *Pangasianodon hypophthalmus* for a period of 96 h.

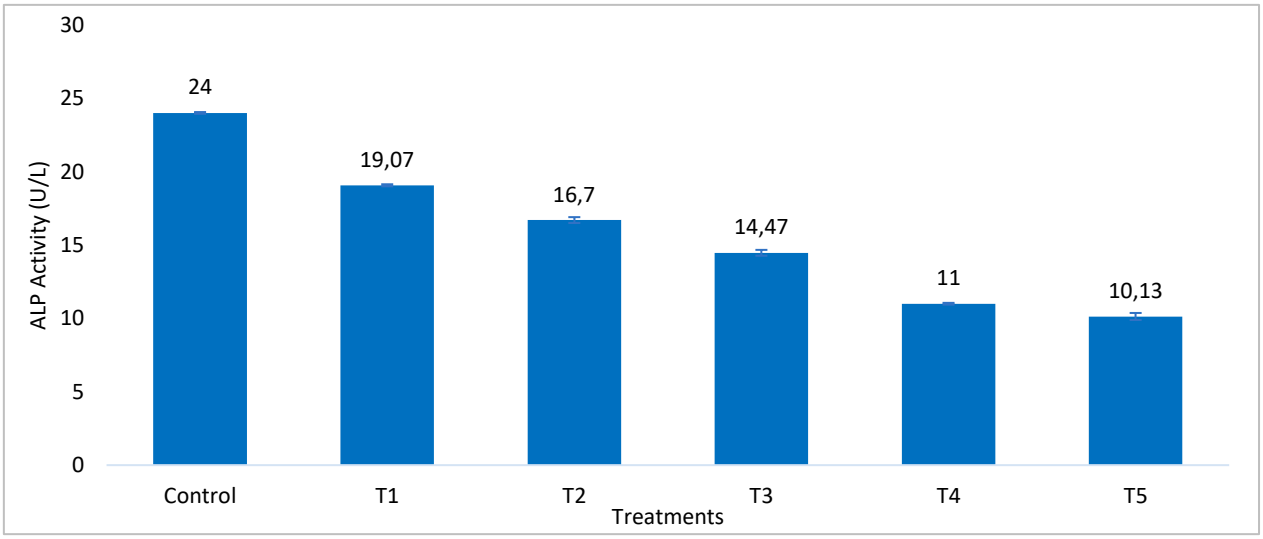

Figure 3. The effects of chlorpyrifos on the serum ALP levels of *Pangasianodon hypophthalmus* for a period of 96 h.

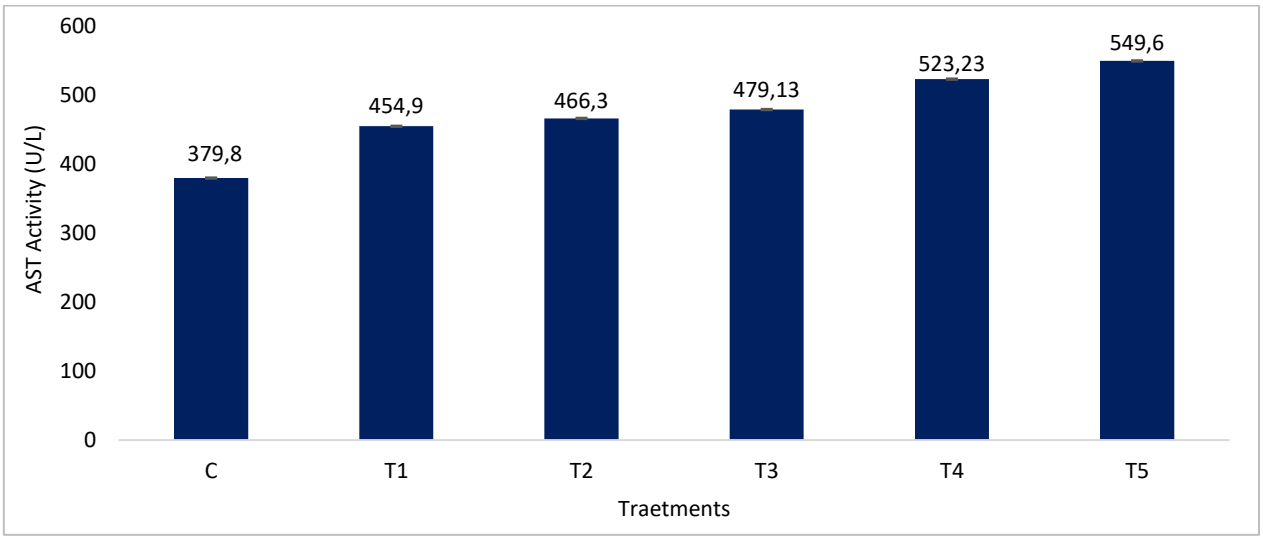

Figure 4. The effects of chlorpyrifos on the AST levels of *Pangasianodon hypophthalmus*.

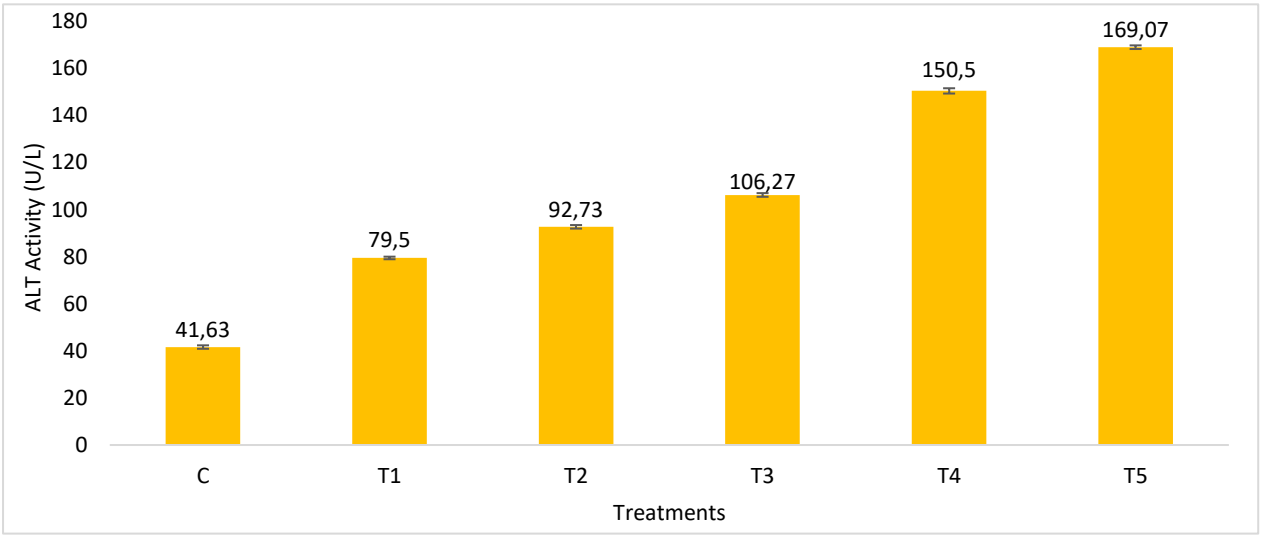

Figure 5. The effects of chlorpyrifos on the ALT levels of *Pangasianodon hypophthalmus*.

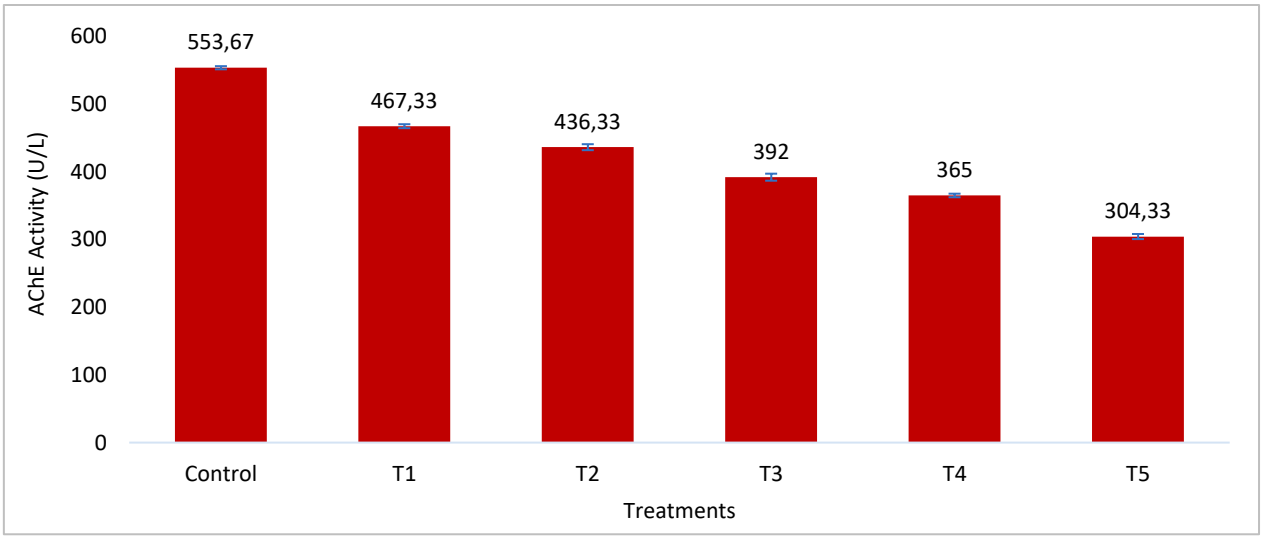

Figure 6. Inhibition of serum AChE activity in *Pangasianodon hypophthalmus* after exposure to chlorpyrifos insecticide at different concentrations for 96 h.

hypophthalmus is represented in Figure 9 (A-F), providing insights into observed changes: (A) Control (0.0 mg L^{-1}): the kidney exhibited normal architecture of renal tubules, glomerulus, bowman's capsule, hematopoietic tissue and occurrence of vacuolation. (B) 0.09 mg L^{-1} CPF exposed group: Signs of exposure include a reduced lumen of renal tubules, increased Bowman space, and focal loss of renal tubules. (C) 0.1 mg L^{-1} CPF exposed group: Features observed consist of hypertrophied hepatocytes and hydropic swelling. (D) 0.11 mg L^{-1} CPF exposed group: Notable alterations include glomerulus shrinkage, pyknosis, hyperplasia, mild vacuolation, and a collapsed glomerulus. (E) 0.12 mg L^{-1} CPF exposed group: The kidney shows necrosis, severe focal loss of renal tubules, increased desquamation, degeneration of renal tubules, degeneration of blood vessels, and degeneration of glomerulus. (F) 0.13 mg L^{-1} CPF exposed group: Showed massive necrosis, degradation of renal tubules, pronounced vacuolation, tubular necrosis and massive melanomacrophages.

In summary, with the increase of concentration of CPF, the kidneys exhibited progressive histopathological alterations, varying from lumen reduction and focal loss of tubules to vacuolation, severe necrosis and cellular degeneration. The findings emphasize the dose-dependent effect of acute CPF exposure on the renal histology of *P. hypophthalmus.*

Discussion

Acute Toxicity

In this study, the LC_{50}-96h for Chlorpyrifos was determined to be 0.106 mg L^{-1} when *P. hypophthalmus* fingerlings were exposed. A thorough review of toxicity

Table 2. Changes in *Pangasianodon hypophthalmus* biochemical parameters after 96 hours of chlorpyrifos exposure to different treatment (concentration).

	Total Protein (g/dL)	ALB (g/dL)	GLB (g/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)
Control	3.80±.06^{a}	1.60±.06^{a}	2.20±.12^{a}	74.33±1.20^{a}	39.57±.43^{a}
T1	3.33±.03^{b}	1.17±.03^{b}	2.17±.03^{a}	61.33±.88^{b}	44.57±.30^{b}
T2	3.07±.03^{b}	0.93±.03^{c}	2.13±.07^{a}	55.00±.58^{c}	48.77±.176^{c}
T3	2.73±.03^{c}	0.77±.03^{cd}	1.97±.07^{a}	51.33±.33^{c}	52.13±.15^{d}
T4	2.27±.09^{d}	0.70±.06^{d}	1.57±.09^{b}	47.00±.58^{d}	57.50±.21^{e}
T5	1.63±.09^{e}	0.57±.07^{d}	1.07±0.03^{c}	40.33±.89^{e}	63.03±.90^{f}

Values in the same column with different superscript differ significantly ($p<0.05$). Data expressed as Mean ± SE (n=6).

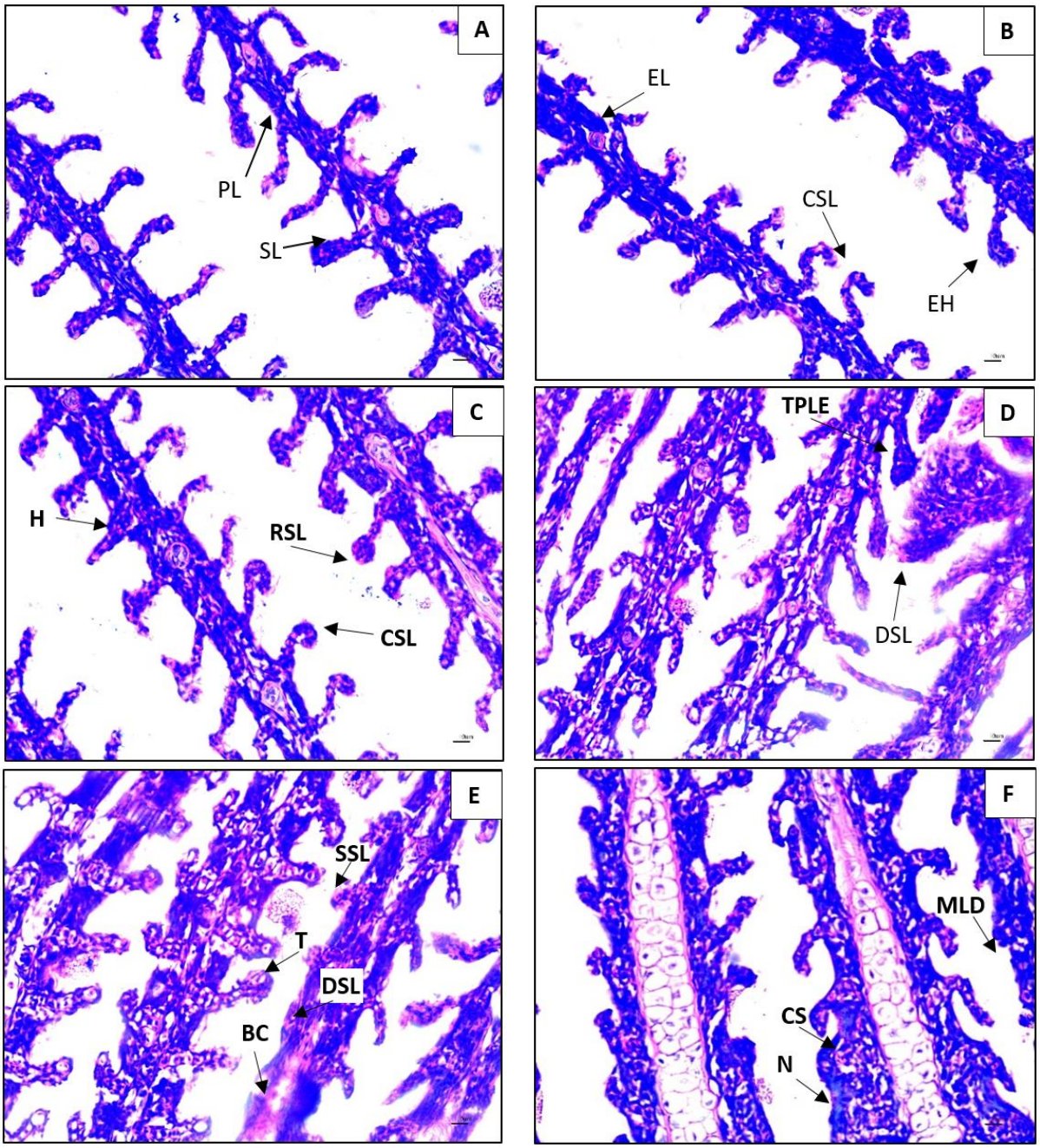

Figure 7. Light micrographs of H & E stained sections of gill of *Pangasianodon hypophthalmus*. (A) Control group showing normal structure (7B -F) treated group gills showing various histopathological alteration including curling of secondary lamellae (CSL), epithelium lifting (EL), epithelial hyperplasia in secondry lamellae (EH), epithelial hyperplasia in primary lamellae (H) and rounding of secondary lamellae (RSL), desquamation of secondary lamellae (DSL), thickening of primary lamellar epithelium (TPLE), shorting of secondary lamellae (SSL), Telangiectasia (T), Degeneration of secondary lamellae (DSL), blood congestion (BC), necrosis (N), collapsed secondary lamellae (CS), massive lamellae degeneration (MLD. (All figures were taken at 40x magnification, and Bars=10µm (except control at 20x).

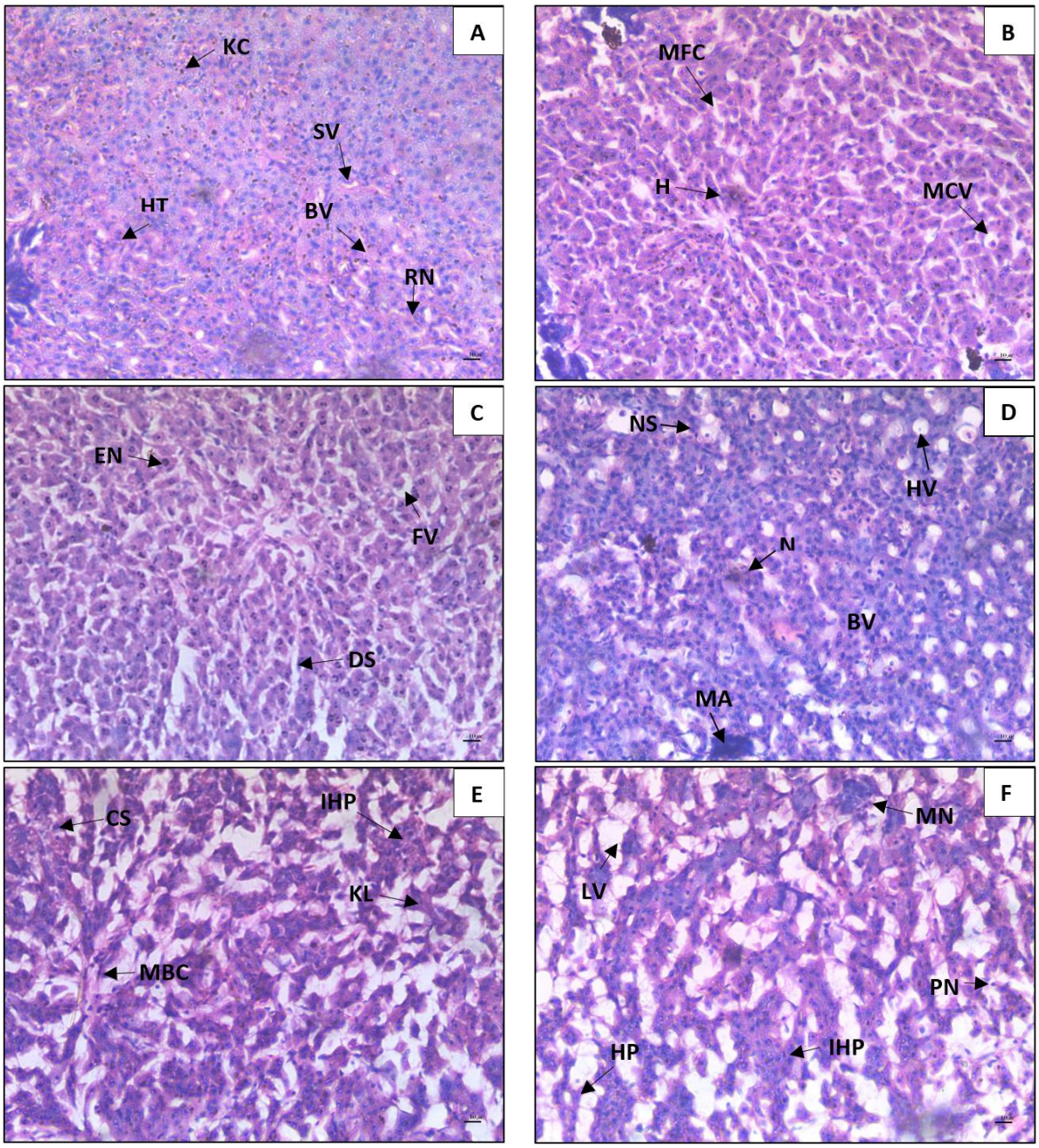

Figure 8. A-F: Light micrographs of H & E stained sections of liver of *Pangasianodon hypophthalmus*). (A) Control group showing normal structure. (B-F) treated groups liver showing various histopathological alterations including minor fatty changes (MFC), hemosiderin (H), mild cytoplasmic vacuolation (MCV), enlarged nuclei (EN), Dilation of sinusoids(DS), fatty vacuolation, nuclei shrinkage (NS), Necrosis (N), Hepatocytes vacuolation (HV) and malanomacrophages aggregation (MA), irregular hepatic plate (IHP), karyolysis (KL), cloudy swelling (CS), mild blood congestion (MBC), massive necrosis (MN), pyknotic nucleus (PN), (HP) hypertrophy of hepatocyte and large vaculation (LV). (All figures were taken at 40x magnification and Bars= 10µm).

data reveals varying 96-hour LC_{50} values for different fish species stating that dosage and responses are species specific. For *Clarias batrachus, Oreochromis niloticus* and *Clarias gariepinus,* the LC_{50} values reported were 0.165 mg L^{-1}, 46.8 µg L^{-1} and 0.861 mg L^{-1} respectively (Narra et al., 2017; Hossain et al., 2022; Nwani et al., 2013). The variations in toxicity levels among different species may be attributed to multiple and diverse factors like differences in the absorption rate, accumulation level, biotransformation, and excretion mechanisms. These variations are due to the complex interactions of biological and environmental factors which influence the toxicity (Al-Ghanim, 2012). Moreover, variations in testing methods, species-specific sensitivity and distinctions in the toxicant's properties may also contribute to the diversity in findings (Dwyer et al., 2005).

Its very important to know the complex nature of pesticides toxicity, where multiple external and internal factors influence the response of aquatic organisms. This comprehension improves the contextual interpretation of LC_{50} values and helps to better understand the ecological risk assessments in relation to pesticide exposure and species specificity.

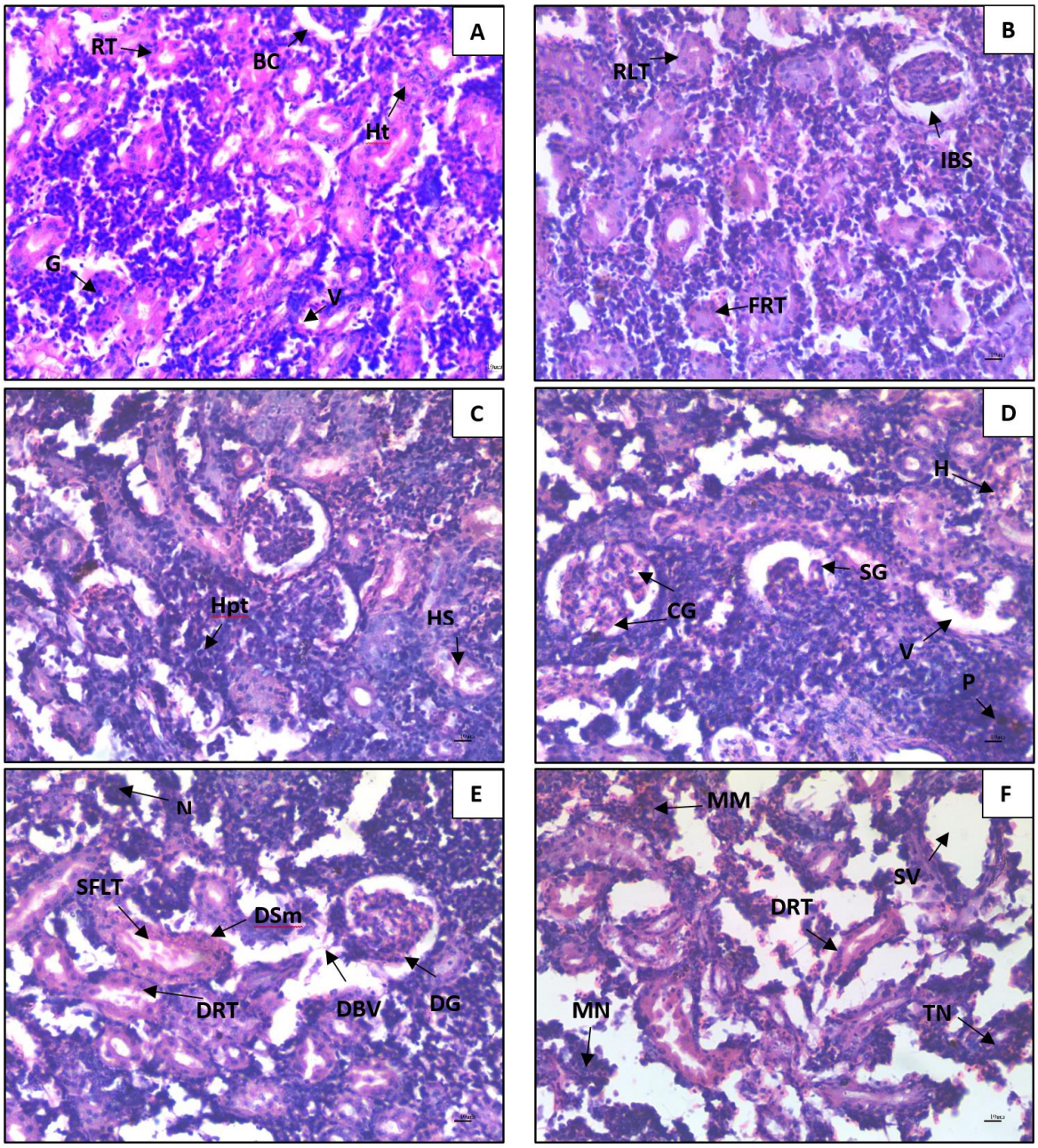

Figure 9. A-F: Light micrographs of H & E stained sections of kidney of *Pangasianodon hypophthalmus*. Control group showing normal architecture of kidney. (B-F) treated group kidney showing various histopathological alteration in kidney including reduced lumen of renal tubule(RLT), increase bowman space (IBS), focal loss of renal tubules (FRT), hypertrophied hepatocytes (Hpt), hydrophic swelling (HS), glomerulus shrinkage (SG), pyknosis (P), hyperplasia (H), mild vaculation (V) and collapsed glomerulus (CG), necrosis (N), severer focal loss of renal tubules (SFLT), desqulation more (DSm), degeneration renal tubule (DRT), degeneration of blood vessels (DBV) degeneration glomerulus (DG), massive necrosis (MN), severer vacuolation (SV), massive malanomacrophage (MM), tubular necrosis (TN). (All figures were taken at 40x magnification and Bars= 10µm).

Biochemical Analysis

The physiological disturbances caused by pathological or chemical stress can be predicted with the help of biochemical profile of blood (Velisek et al., 2009). In the present study, the manifestation of chlorpyrifos as a stressor to the test fish is clearly elucidated, as evidenced by a substantial increase in glucose concentration corresponding to elevated pesticide concentrations. Given that carbohydrates represent the major and most direct energy source, the noticable rise in plasma glucose is widely recognized as a sensitive indicator of environmental stress in fish (Nemcsok and Boross, 1982). Similar results were found in other species, such as *Clarias batrachus* (Narra et al., 2015) and *Pangasianodon hypophthalmus* (Islam et al., 2019).

These observations were further supported by Banaee et al. (2011) attributing the increased levels of blood glucose in fish exposed to chlorpyrifos, possibly associated with the increased energy requirements. CPF exposed fish also exhibited significant reduction in

serum protein levels, including albumin and globulin. The decreased levels of albumin and globulin may result from a decrease in plasma total protein levels, possibly due to stress-induced mobilization of substances to overcome the elevated energy demand. This is consistent with the significantly decreased serum protein, globulin and albumin levels observed when exposed to chlorpyrifos in this present investigation. Such reductions in protein levels are reminiscent of conditions like starvation, malabsorption, and malnutrition (Bhatnagar et al., 2017). Similar findings were observed in common carp (*C. carpio*) exposed to chlorpyrifos (Hatami et al., 2019) and *Oncorhynchus mykiss* exposed to bifenthrin (Velisek et al., 2009). But a significant decrease in triglyceride levels in fish serum was observed on exposure to CPF. This can be attributed to decreased absorption efficiency in the intestine, interruptions in liver biosynthesis and malnutrition caused on exposure to CPF (Hatami et al., 2019).

In conclusion, this study provides a brief insight into the blood biochemical profile with a clear understanding on stress-induction by CPF on the test animal, which is supported by various findings in other fish species. This study also helps to understand the diverse effects of CPF on metabolic pathways and emphasizes the significance of considering these effects on aquatic ecosystem in relation to environmental stress.

Haematological Studies

Hematological characteristics helps to understand normal, pathological and as well as the toxic impacts on fish (Velisek et al., 2009). In the context of this study, exposure to chlorpyrifos (CPF) resulted in a significant decline in RBC, Ht and Hb levels alongside an elevation in white blood cell (WBC) count. Similar observations were reported by Ismail et al. (2018), Ali et al. (2020), and Ural et al. (2013) in rohu, *Oncorhynchus mykiss*, and common carp, respectively when exposed to CPF.

Vani et al. (2011) stated that the decreased RBC and Hb levels could be due to the inhibition of processes like erythropoiesis, haemosynthesis, dysfunction of osmoregulation or decomposition of erythrocyte in the hematopoietic organ. In the present study, the reductions observed in hematological parameters reveals the potential impact of chlorpyrifos on crucial processes of hematopoietic system.

The white blood cells (WBC) count also increased upon exposure to CPF, indicating stimulation of the leucopoietic system to produce excess white blood cells (Ghayyur et al., 2019). Stress caused by CPF might be the reason for the increased white blood cells (WBC) count, as the immune system gets activated to counter the potential threats.

Changes in blood cell indices were also observed in this study, including mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV). These changes could be due to the high sensitivity of these indices, which can irreversibly alter fish homeostasis. The changes noticed in these indices are directly corelated with the alterations in packed cell volume, hemoglobin concentration, and RBC count, further stating the effect of chlorpyrifos on fish hematological parameters.

In summary, the hematological alterations recorded in the present study provide a clear understanding of the toxic effects of chlorpyrifos on fish, affecting both erythrocyte and leukocyte parameters. The findings are consistent with the previous studies, stating the importance of hematological indices as indicators of stress and potential toxicity in aquatic organisms.

Enzyme Activity

In this study, it was noticed that, exposure of *P. hypophthalmus* to CPF significantly elevated the serum alanine aminotransferase and aspartate aminotransferase activities. These elevations in enzyme activity indicate hepatocellular damage or liver necrosis caused by chlorpyrifos exposure (Borges et al., 2007). CPF toxicity effect on enzyme activity may be linked to the cellular deterioration, potentially leading damage to vital organs such as the heart, brain, or muscles (Raibeemol and Chitra, 2018). Consistent results were found in other species, such as *Cyprinus carpio* (Banaee et al., 2013; Hatami et al., 2019) and *Caspian trout* (Adel et al., 2017) upon exposure to chlorpyrifos.

In addition to ALT and AST, the study reported alterations in serum alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in *P. hypophthalmus* after exposure to acute chlorpyrifos doses. Specifically, ALP activity decreased, while ACP activity increased. In *Clarias batrachus*, similar effects on ALP (Narra et al., 2011) and for ACP in *Gambusia affinis* (Khan and Sharma, 2012), were reported on exposition to chlorpyrifos. For metabolism of carbohydrates and transportation of phosphorylated intermediates across the membranes of cells, Alkaline phosphatase (ALP) plays a vital role (Vijayavel and Balasubramanian, 2006). The decrease in ALP activity may signify hepatocellular injury induced by chlorpyrifos. On the other hand, the increase in ACP activity could be linked to the response of the organism to the toxicant.

The acetylcholinesterase (AChE) helps in breaking down the neurotransmitter acetyicholine (Ach) to ensure proper nervous system functioning. In this study, it was observed that the AChE activity decreased in the toxic treated groups in comparison to the control group. Similar results were documented in various fish species by various authors in *Rutilus caspicus*, *M. nipponense*, *Clarias gariepinus*, and *Cyprinus carpio* (Zahmatkesh et al., 2020; Hong et al., 2018; Harabawy and Ibrahim, 2014; Yonar et al., 2014; Hatami et al., 2019). This reduction in AChE activity demonstrates the adverse effect of CPF on inhibiting serum AChE activity. The

inhibition of AChE leads to the accumulation of free Ach causing continuous electrical stimulation and nervous system degradation (Baldissera et al., 2021).

In summary, the findings of this study emphasize the impact of CPF on biochemical markers, particularly enzymes associated with liver and nervous system functioning in *P. hypophthalmus*. These findings also help to better understand the toxicological effects of CPF on the aquatic organisms.

Histopathological Alteration

Gills are the primary respiratory organs along with respiration they play a vital role in osmotic and ionic regulation, acid-base equilibrium, and the excretion of nitrogen (Evans et al., 2005). Expansive surface area of gills and other morphological characters makes them susceptible to dissolved contaminants and a significant avenue for environmental impact (Fernandes, 2019).

In the context of the current studies, the architecture of gill in all treated groups displayed notable histopathological changes. These alterations closely resemble the effects induced by cypermethrin exposure in *C. catla* over fifteen, thirty and forty five days of treatment periods at concentrations of 0.12 µg L^{-1} (Sharma and Jindal, 2020). The extensive surface area facilitates the fish to absorb pollutants and the short distance for water-blood diffusion is important for oxygen uptake in aerobic metabolism (Fernandes and Moron, 2020).

Sepici-Dinçel et al. (2009) state that the increasing distance between the external environment and blood acts as a barrier to the entry of pollutants/contaminants, exhibiting changes in the structure of gill such as lamellar fusion, epithelial lifting, and hyperplasia of epithelial cells. The entry of these contaminants into the bloodstream can be prevented by lifting the epithelium to increase the distance. Edema-induced lamellar lifting, as proposed by Schwaiger et al. (2004), contributes to the observed histopathological changes.

On exposure to CPF, the gills exhibited potential effects for oxygen consumption and disruption of osmoregulatory activities. Respiration and osmoregulation are the primary activities performed by gills and histological lesions such as lamellar fusion and epithelial lifting reduce the oxygen absorption efficiency by secondary lamellae. This reduction in oxygen uptake efficiency can induce respiratory distress, subsequently hindering fish activity and growth (Caldwell, 1997).

Liver is a vital organ performing many vital physiological processes, including metabolism, homeostasis and synthesizes numerous enzymes. Liver is also a key target organ for histological biomarkers used in investigating of liver damage (Ghayyur et al., 2021; Sharma et al., 2019). In the present study, a number of histological abnormalities were observed in the liver, indicating potential damage. These abnormalities included hemosiderin accumulation, mild cytoplasmic vacuolation, elongation of nuclei, dilation of sinusoidal, fatty vacuolation, shrinkage of nuclei, aggregation of malanomacrophages, karyolysis, cloudy swelling, mild blood congestion, massive necrosis, pyknotic nucleus, and hypertrophy of hepatocytes. These changes were similar with the findings in *Heteropneustes fossilis* exposed to organophosphate envoy (Akter et al., 2020) and *Channa Punctatus* treated with hexavalent chromium (Mishra and Mohanty, 2008).

Farhan et al. (2021) also reported multiple changes in liver tissue on exposure to CPF, including cell degeneration, necrosis, and hemorrhage. The present findings are compatible with Magar and Shaikh (2013), who observed similar changes in *C. punctatus* on exposure to Malathion for four days. The hepatocyte vacuolization may suggest an imbalance between synthesis and substance release emphasizing the potential impairment of liver function caused by the toxicant.

Necrosis may be induced in the liver by elevated levels of chlorpyrifos and causing degeneration of hepatic cells and also the liver loses the regenerative capacity in the presence of toxicants. The lesions observed in liver result from insufficient oxygen caused by gill degeneration, compounded by hemolysis in blood vessels (Suchana et al., 2020).

In this study, the histological abnormalities observed in liver indicate significant liver damage, which is consistent with many studies conducted on various toxicant exposures. To assess the impact of CPF on aquatic organisms, it is very important to understand these alterations in liver.

Homeostasis is maintained by kidneys in fish by eliminating waste from the blood, selective reabsorption and regulating blood volume, pH and erythropoiesis. So, its proper functioning is important for maintaining a good health (Iqbal et al., 2004). The alterations observed in this study are parallel to the findings reported by Cengiz (2006), who documented degeneration of pyknotic nuclei and epithelial cells of hematopoietic tissue and renal tubules respectively, along with glomerular deterioration in deltamethrin exposed fishes (Gill et al., 1989). Similarly, Staicu et al. (2008) reported various abnormalities, including cytoplasmic vacuolization, alterations in cell and nuclear volume, necrosis of renal tubule, nuclear malformations, nuclei pycnosis, anisogamy and hypertrophy of nuclei in *Carassius carassius* when exposed to 0.05 mg L^{-1} of Malathion for a duration of 72 hours. The dynamic processes caused by CPF, such as vascular and exudative stages, may lead to necrosis and kidney degeneration in *P. hypophthalmus.*

Renal lesions act as reliable indicators of environmental pollution, as most of the post-branchial blood is received by kidney. Palermo et al. (2015) stated that disrupted glomerular filtration activity and impaired excretion may lead to the accumulation of toxicants in the kidney, causing organic failure. The histopathological observations made in this study suggests that exposure of *P. hypophthalmus* to CPF at

lethal concentrations causes detrimental effects on gill, liver, and kidney tissues.

Based on the present and pervious findings, the histopathological alterations observed in gill, liver and kidney tissue may arise from severe physiological problems, leading to the death of the fish. These findings highlight the significance of understanding the impact of environmental contaminants on the complex physiological processes of aquatic organisms, emphasizing the urgency of effective conservation and pollution control measures.

Conclusion

The findings of this study are in conclusion that the commonly used agricultural pesticides like chlorpyrifos negatively effects the histology and biochemical hematological parameters of non-target aquatic species like *P. hypophthalmus.* The findings highlight the need for careful consideration and meticulous planning on the use of chlorpyrifos to prevent adverse impacts on the aquatic ecosystem. The results also helps in assessing ecological risk and uncertainties emphasize the importance of adopting a cautious and ecologically responsible approach in pesticide application, sustainable practices and regulatory measures to safeguard non-target species and preserve aquatic organisms health.

Ethical Statement

The fish management and sample procedures were carried out in accordance with the animal care protocols (No. COF/FHY/BOS/783/2022) approved by the College of Fisheries, Ratnagiri (Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India) Committee on Animal Ethics.

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Author Contribution

B.C.: Conceptualization, Investigation, Methodology, Formal analysis, Original draft preparation, Writing- Reviewing and Editing. **H.B.D.**: Supervision, Methodology, Validation, Writing- Reviewing and Editing. **A.S.P.**: Supervision, Data curation, Writing- Reviewing and Editing. **P.H.S.**: Supervision, Methodology, Validation and Editing. **B.R.C.**: Supervision, Data curation, Writing- Reviewing and Editing. **S.J.M.**: Conceptualization, Investigation, Methodology, Validation, Data curation. **M.K.**: Conceptualization, Methodology, Software. **E.A.G.**: Conceptualization, Methodology, Validation, Software.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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